

3457-Pos**Ceramide Gel Domain Formation in a Phospholipid Bilayer: The Impact of Ceramide Acyl Chain**Sandra N. Pinto¹, Liana C. Silva^{1,2}, Rodrigo F.M. de Almeida³, Manuel Prieto¹.¹CQFM & IN, Instituto Superior Técnico, Lisboa, Portugal, ²Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel, ³DQB, FCUL, Lisboa, Portugal.

Nowadays, it is recognized the critical role of Ceramide (Cer) in signalling pathways that leads to cell proliferation, apoptosis, growth and inflammation. It is believed that the biological action of Cer depends largely on its biophysical properties, e.g. the formation of Cer platforms in cell membranes was suggested to lead to the activation of apoptotic signaling pathways. Moreover, it has been proposed that Cer containing different fatty acids have distinct impact upon cell physiology. Regarding these observations, the biophysical behaviour of long and saturated (C16:0, C18:0) and very long and unsaturated (C24:1) acyl chain Cer in a fluid membrane (palmitoyl-oleoyl-phosphatidylcholine (POPC)) was studied and compared.

The application of i) fluorescent spectroscopy and ii) confocal fluorescence microscopy showed that Cer acyl chain is the limiting factor on Cer/fluid phospholipid interaction. For instance, at room temperature (24°C) at least 10% of C24:1 Cer are needed to observe lateral segregation, in contrast only 4% of C16:0 and C18:0 Cer are required for microdomains formation. From confocal fluorescent microscopy complex tubular structure, known as cochleate, were only observed for bilayers composed of small amounts of C24:1 Cer (20-30%). Moreover the used of fluorescence correlation spectroscopy for studying N-rhodamine-dipalmitoylphosphatidylethanolamine diffusion in giant unilamellar vesicles (Cer/POPC), revealed a higher capacity of C16:0 and C18:0 for ordering fluid areas surrounding Cer platforms. Altogether, these observations support the suggestion that each Cer are involved in distinct cellular processes and membrane physical properties are a key mechanism for cell metabolism regulation.

3458-Pos**Probing Cholesterol Dependent Lipid Properties Using Molecular Dynamics Simulations of BODIPY-PC in Explicit DMPC and DPPC Membranes**

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Dunn and co-workers have used polarized total internal reflection fluorescence microscopy to measure the tilt angle of a single molecule (BODIPY-PC) relative to the membrane normal to characterize the membrane properties. Recently, they found that increasing quantities of cholesterol in monolayer and bilayer membrane systems leads to a much smaller tilt angle of the BODIPY-PC molecule. To better understand the influence of cholesterol on membrane properties and its relation to BODIPY-PC tilting, we have performed molecular dynamics (MD) simulations of BODIPY-PC in explicit dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphocholine (DMPC) monolayer and bilayer membrane systems with systematically increasing concentrations of cholesterol (0, 5, 10, 15, 20, 33, 50%). After 4 ns of equilibration, each system has been simulated for another 16 ns. Preliminary results indicate that the BODIPY-PC tilt angle decreases as a function of increasing cholesterol concentration until 10% cholesterol after which there appears to be cholesterol saturation and the rigidifying affect ceases. Other points of interest include confirming the applicability of monolayer data for bilayer systems and explaining any quantifiable differences between the DPPC and DMPC membrane systems. We will present the orientation (azimuthal and rotational angle distribution) of BODIPY-PC, the BODIPY-PC tilting energetics, various membrane properties, and the spatial distribution of the cholesterol as a function of cholesterol concentration.

3459-Pos**Dynamics of Phase Separation in Lipid Membranes**

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We present a continuum method to simulate the large-scale dynamics of multicomponent model membranes, as observed in various recent fluorescence microscopy experiments. Our model includes both thermal fluctuations and the quasi-two-dimensional hydrodynamics appropriate to a membrane immersed in an outside fluid, which are crucially important for the simulation of membrane domain flickering. Using this coarse-grained scheme allows us to simulate length scales up to tens of microns, and time scales of seconds. We apply our model to phase separation, domain fluctuations, and diffusion of domains in lipid systems, and show agreement between our simulations, experiments, and theories for known limiting cases.

3460-Pos**Molecular Dynamics Simulations of In-Plane Density Fluctuations in Phospholipid Bilayers**

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At room temperature many lipids form the bilayer structure of biological membranes, which resembles liquid crystals. The structural features of the fluid lipid bilayer are well-known, but many details of the dynamic behavior of biological membranes still remain to be understood.

Molecular motions in liquids can be monitored by the intermediate scattering function, $F(q,t)$. In membranes it probes the propagation and decay of in-plane density fluctuations at wave vector q . An attractive property of the intermediate scattering function is that it can equally well be determined from scattering experiments as from molecular dynamics simulations, opening the possibility of direct comparison between experimental and simulation data. Density fluctuations in lipid bilayers can stay correlated for hundreds of nanoseconds which implies that in contrast to simple liquids, an exponential decay of $F(q,t)$ as suggested by a purely single diffusive model, does not describe how fluid membranes behave. Microsecond molecular dynamics simulations on thousands of lipids, both atomistic and coarse-grained, has been used to explore $F(q,t)$. The atomistic simulations span membrane patches of tens of nanometers while the coarse-grained simulations, including almost 100 000 lipids, reach the low micrometer domain. The main purpose was to establish a dispersion relation for the density fluctuations, i.e. a relation between the wave vector and the decay rate. Different model functions are compared to find the dispersion relation that best fits the simulation data. The important question of how material properties (bending modulus and area compressibility) are related to the molecular structure of the complex liquid is addressed in the context of the different models.

3461-Pos**Age Dependent Deformability Changes of RBCS Evaluated Using a Cyclically Reversing Shear Flow Generator**Naoyuki Yokoyama¹, Daisuke Sakota¹, Toshitaka Yasuda², Setsuo Takatani¹.¹Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan, ²Tokyo Technical High School of Engineering, Tokyo, Japan.

In this study, using a cyclically reversing shear flow generator we studied deformability of blood cells separated into young and old RBCs. We investigated the relationships between aging of RBCs and its deformability sheared by a cyclically reversing flow.

To study the effect of aging of cells on their mechanical properties, RBCs were separated by centrifugation according to their density. The sampled RBCs were then mixed with Dextran solution to prepare 0.15% Hematocrit RBC suspension.

The RBC suspension was placed between the two parallel glass plates separated by a gap distance of 30 μ m under a microscope; with the bottom plate stationary, the upper plate made a reciprocal motion by cam-motor machinery (the amplitude and frequency of 1.25mm and 2, 4, and 6 Hz, respectively) creating a quasi-Couette flow of RBC suspension with the peak shear stress of 37.46 Pa. The images of RBCs were magnified by a $\times 40$ objective lens combined with a $\times 1.6$ middle lens, and recorded by a high speed video camera. Subsequently, the RBCs images have gone through averaging, digitization, and then morphological analysis of their properties in terms of the major (L) and minor (W) axes of the projected two dimensional figures, yielding the deformation index of L/W .

The L/W of both groups of RBCs followed closely the shear stress changes, exhibiting asymmetrical response patterns consisting of fast deformation or stretching and slow recovery phase. The mean L/W of young RBCs was 1.096 ± 0.0079 times that of the old ones ($p=0.0003$).

Our preliminary study indicated more rigid membrane properties in the old RBCs as compared with the young cells at three different shearing frequencies.

3462-Pos**Biomimetic Metallic Electrodes for Intracellular Electrical Measurements**

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Electrical interfaces represent a powerful approach for interrogating or perturbing biological systems, with applications in neural prostheses, the regulation of artificial neuronal networks and arrayed patch clamp diagnostics. A key step towards achieving this interface is the development of inorganic nanostructures that can specifically and non-destructively incorporate into biological membranes. Here, we report the development of nanoscale metallic posts that mimic transmembrane proteins, allowing their spontaneous insertion into lipid membranes. These electrode posts were formed by metal evaporation with